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BIOLOGICAL BULLETIN

STUDIES ON THE RELATION BETWEEN AMITOSIS AND MITOSIS

II. Development of the Testes and Spermatogenesis in Moniezia (*Continued*).

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IV. *The Spermatocytic Mitoses.*

The appearance of the chromosomes of the first maturation division follows the stage shown in Fig. 13, *A* (Pl. X.). Figs. 14, *A*, 14, *B*, and 15 (Pl. XI.) show this stage in *M. expansa*, Figs. 16, *A*–16, *C* (Pl. XI.), in *M. planissima*. In the former species eight of these chromosomes have been counted in the nuclei in some twenty-five cases (Fig. 14, *A*) and in no case have more than eight been found. In many cases, however, it has been impossible even with the utmost care in examining successive sections, to find eight, only seven or six (Fig. 14, *B*) being visible. As regards the number in *M. planissima* the results are less definite. In some cases eight (one of the nuclei in Fig. 16, *C*) in others nine (the other nucleus in Fig. 16, *C*) have been counted and in one (Fig. 16, *B*) case thirteen distinct masses of chromatin were visible in one nucleus. This, however, was probably an earlier stage for some of these masses appear to be grouped in pairs. Fig. 16, *A*, shows two nuclei of *M. planissima* in which five of these chromosomes are visible. It was impossible to determine whether parts of these nuclei were in the next section. One reason for the greater uncertainty in regard to *M. planissima* lies in the fact that these stages were much less frequently seen in my sections of this species than in those of *M. expansa*. I believe that the utmost caution should be used in observations of

this kind. In a study of cytological literature it is difficult to resist the impression that some of the apparent uniformity in cytological phenomena which appears so remarkable and mysterious is in reality the result of the selection of certain "typical" cases and the discarding of others. While I should not venture to assert positively that the number of chromosomes in either of these species of *Moniezia* is actually variable, this certainly appears to be the case, and the evidence given in support of the conclusion that the number of chromosomes in a given species or variety is invariable has not always carried conviction to my mind.

However, the description of the spermatocytic divisons is not the chief object of the present paper and although I have spent much time in endeavoring to reach well-founded conclusions in regard to the number of chromosomes in *Moniezia* it has not been possible for *M. planissima*. In *M. expansa*, as noted above, the maximal number counted during maturation was eight.

In some of the nuclei at this stage these chromosomes appear, as in many other forms in a more or less regular grouping about the periphery of the nucleus. The figures do not show this condition particularly well but Figs. 14, *A*, 14, *B*, 16, *A*, 16, *C* (Pl. XI.), were drawn from nuclei in which the chromosomes were thus arranged. Such nuclei are recognizable at once in a section. This condition, however, was not very commonly found; much more frequent was the condition shown in Fig. 15 where they were irregularly disposed, some at the periphery, others near the center. It is possible that the stage of peripheral arrangement is of relatively short duration and is therefore less frequently seen, but there is also a possibility that it is not of universal occurrence. The question may be left open for the present.

In Fig. 17 (Pl. XI.) two of these chromosomes are shown, both from nuclei with membranes still intact. At the stage figured the fusion of the two parts is not yet complete. So far as actual observation goes these chromosomes of the first maturation division are dyads, not tetrads, no indication of quadrivalence having been observed at any stage, and although they correspond as regards ultimate fate with the tetrads of various authors, I prefer to designate them as what they appear to be — dyads. In consequence of their small size, almost spherical form in late stages,

and close approximation in the spindle no data regarding the direction of the two divisions could be obtained.

As stated in the preceding section the spermatocytes often appear in groups, the pear-shaped members of which are connected at the pointed end by strands of cytoplasm. As the first spindle forms the fusion of the cells proceeds (Fig. 18, Pl. XI.) until in many cases the metaphases of a number of nuclei lie in a continuous mass of cytoplasm, the cytophore (Fig. 19, Pl. XI.). Often, however, the central region of the mass consists for a considerable time of a large space traversed by strands of cytoplasm (Figs. 18, 20, 23 (Pl. XI.), 25 (Pl. XII.). The strands of cytoplasm are represented in a somewhat diagrammatic manner.) In other cases the first spermatocytes are entirely isolated (Fig. 21, Pl. 11).

The spindle appears to be formed largely from the nuclear substance. The fibers are very delicate and no asters are visible (Figs. 18 and 19, Pl. XI.). At the poles are very minute, but fairly distinct deeply-staining, centrosomes. In the masses resulting from the fusion of several spermatocytes the spindles are, so far as observed, always tangential or nearly so (Figs. 19 and 20, Pl. XI.). Just before their division the dyads assume the form seen in Fig. 22 (Pl. XI.), where one is viewed from the side, the other from the surface. Fig. 20 (Pl. XI.) shows the anaphase. Division of the centrosomes has not been observed, as they cannot be distinguished after the chromosomes have approached the poles. Fig. 23 (Pl. XI.) shows the telophase of the first spermatocytic division in a group. The cytoplasm of the cells which formed the group now constitutes a cytophore in which the masses of chromatin lie. It is less dense in appearance and stains less deeply than in earlier stages, where it was concentrated about particular nuclei. The chromatin masses remain at the periphery and the central region is often still more or less vacuolated. Where isolated spermatocytes divide the result is the same: no division of the cytoplasm follows nuclear division, and a cytophore differing from the group-cytophore merely as regards size is formed.

The second division follows the first, in most cases apparently without the formation of a "resting nucleus" between the two. In a few cases, however, nuclei larger than spermatid nuclei and

containing irregular strands and masses of chromatin as if in preparation for a division were formed about the periphery of a cytophore (Fig. 24, Pl. XI.). These were very probably stages following the first spermatocytic division.

Fig. 25 (Pl. XII.) shows the metaphase of the second spermatocytic division in a group cytophore. Here again the divisions are more or less nearly tangential. Fig. 26 (Pl. XII.) shows the anaphase in a portion of a group.

V. *The Fragmentation of Spermatocyte Nuclei.*

In Section III. (pp. 181–182) it was stated that certain of the nuclei of the first spermatocytes become larger than the others, and that the spireme instead of becoming more dense and giving rise to chromosomes becomes less dense and very irregular and the nucleolus remains instead of disappearing (Fig. 13, *B*, Pl. X.). These nuclei constitute the earliest recognizable stage in a remarkable process of fragmentation which is apparently a normal phenomenon in the testes of both species of *Moniezia*. The process is so entirely different from anything described in the spermatogenesis of other forms that at first I regarded it as a form of degeneration. But repeated examination of old and new material during four successive years has convinced me that the process gives rise to nuclei which are indistinguishable from the spermatid nuclei which have arisen by mitosis. Whether these "spermatid nuclei" resulting from the fragmentation of spermatocyte nuclei actually take part in the formation of functional spermatozoa it is impossible to determine, but that spermatozoa structurally similar to those developing from nuclei which arise in the ordinary manner develop from these spermatids is, as will appear probable.

This process, like the typical spermatogenesis, occurs in isolated cells or simultaneously in groups. In the latter case the different members of the group fuse together, in the manner described in the preceding section, and this cytoplasm forms a cytophore. It is rare, however, that groups so regular in form and arrangement as that shown in Fig. 27, *A* (Pl. XII.), are found More commonly only two or three cells fuse together or the process occurs in isolated cells. Fig. 27, *A* (Pl. XII.) shows a

group of the nuclei in most of which the spireme is apparently breaking up and disappearing. It stains less deeply than in earlier stages and appears to be separating into irregular masses. Fig. 27, *B* (Pl. XII.), shows an exceptionally clear case, in which some portions of the spireme are apparently breaking up into granules. Figs. 27, *C*, and 27, *D* (Pl. XII.), show still other cases. It will be observed that the nucleolus retains its staining power: as a matter of fact it undergoes no visible change.

Except for the spireme or its remnants the nucleus shows with the usual degree of extraction no visible structural features. As it increases in size the nuclear membrane becomes increasingly difficult to distinguish and finally disappears, so that what was formerly the nucleus appears merely as a cavity in the cytoplasm containing the nucleolus and irregular shreds and granules of chromatic substance usually situated at one side (Figs. 27, *B*, 27, *C*, 27, *D*, 28, Pl. XII.). The whole appears as if degenerating. In these and the following figures the boundaries of the nuclear cavities and the spaces in the cytoplasm which remain in the later stages are indicated by broken lines.

But the observations now to be described afford very strong evidence in favor of the view that these nuclei do not undergo complete degeneration. In and about these cavities containing the remains of the spireme and the nucleolus very small new nuclei with one or a few small granules of chromatin appear to be formed. In Fig. 28 (Pl. XII.), one such nucleus is shown in one of the cells: in Figs. 29, *A*, and 29, *B* (Pl. XII.), two appear in each cell: in Fig. 29, *C* (Pl. XII.), is one a little larger than the preceding; in this case the fragmenting nucleus lies in a cytophore with spermatids already formed and from one of which a spermatozoön is developing: in Fig. 29, *D* (Pl. XII.), are again two of the small nuclei. The nature of the process appears to be somewhat as follows: as the old nuclear membrane breaks down the cytoplasm encroaches more or less upon the nuclear cavity and a new membrane forms about some of the particles or masses of chromatin. This is not very different from what occurs in various forms when a single chromosome or a few chromosomes are separated from the rest during division.

But the new nuclei are not always so small as these shown in

Figs. 28 and 29, *A*–29, *D*. Figs. 30, *A*–*C* (Pl. XIII.), show cases in which they are somewhat larger, and in which the method of their formation is more clearly visible. In these cases the new nuclei are nearly hemispherical in form and appear as if growing or budding out from the cavity representing the old nucleus into the cytoplasm about it. Whether the method of formation is actually the same in all cases is doubtful; sometimes the new nuclei seem to lie wholly within the cavity as in Figs. 29, *A* and 29, *B* (Pl. XII.). But there can be no doubt of their formation. The newly formed nuclear membrane is quite distinct and the whole process presents a very characteristic appearance.

The number of deeply staining bodies visible within these small nuclei depends on the fixation and staining. If extraction is carried beyond a certain point only one dark body retains the stain: otherwise several may be visible, one or two of which are usually larger than the others. After chrom-oxalic the stain is more readily extracted from all except the one body and with the usual degree of extraction only the one body appears. Figs. 29, *A*–29, *D*, are from such preparations. In nearly all cases the nuclei stain somewhat more deeply throughout than the cytoplasm as is indicated in the figures by stippling.

Whether the number of new nuclei formed is always the same is uncertain but it is probable that as many as three or four and perhaps more new nuclei may arise from one old nucleus. In Fig. 29, *E* (Pl. XII.), four small nuclei lie near the lower nuclear cavity but some of these may have arisen from another nucleus.

Figs. 31–36 (Pl. XIII.), show what I regard as later stages in this process. In the group shown in Fig. 31 two spermatocytes were apparently involved and the two old nuclear cavities are still visible, though very irregular in form and divided by strands of cytoplasm. One of the old nucleoli is visible, the other lying in another section. Judging from their position three of the small nuclei were formed about the cavity on the right and one on the other: in the next section more small nuclei were found which probably belonged to the nuclear cavity on the left but in this section other adjoining nuclear cavities with other small nuclei appeared, so that certainty was impossible. It is of in-

terest to note that some of the nuclei in the figure are distinctly hemispherical as if they had been formed in the manner represented in Figs. 30, *A*–30, *C*.

In Fig. 32 (Pl. XIII.) three cavities appear, the old nucleolus being visible in the one on the right. In the middle cavity there are four nuclei. Fig. 33 (Pl. XIII.) shows another case in which portions of two nuclear cavities are visible, each with its nucleolus, and seven nuclei, four about one cavity, three about the other.

In Figs. 34, 35 and 36 (Pl. XIII.) groups of nuclei are shown lying in cavities of the cytophore: nucleoli were not visible in this section and it was impossible to determine how many spermatocytes were involved in the formation of these groups. That such groups as these are formed by fragmentation there can, I think, be no doubt. In all cases where the spermatocytes divide mitotically in groups the spindles lie peripherally and nearly tangentially in the cytophore (Figs. 19, 20, 23, Pl. XI.; 25, 26, Pl. XII.), and the nuclei formed lie at or near the periphery (Figs. 24, Pl. XI.; 42, 43, 44, Pl. XIV.). There are no grounds for supposing that they migrate from the periphery and return to it; moreover, it is difficult to see how nuclei grouped as those in Figs. 34 and 35 (Pl. XIII.) are grouped could have been formed *in situ* by mitosis. It seems probable that this difference in position is quite sufficient to distinguish the nuclei formed by fragmentation of spermatocytes from those formed by the spermatogenetic mitoses.

Assuming that these nuclei have arisen by the fragmentation of spermatocytes, the number of chromatin granules has increased beyond that contained in some of the nuclei at the time of their formation and the nuclei are now somewhat larger than some of those. No further growth takes place, however. The nuclei themselves are indistinguishable from those spermatid nuclei which lie at the periphery of the cytophores and are doubtless the products of mitoses (Figs. 42, 43, Pl. XIV.).

Figs. 37 (Pl. XIII.) and 38 (Pl. XIV.) represent larger areas of two testes showing how the nuclei which, judging from their position in the cytophore, have arisen by fragmentation are situated in relation to other stages. No trace of definite position or arrangement of the various stages in the testis could be found.

But these nuclei apparently do not remain massed together in the cytophore. There is every reason to believe that they migrate to the periphery. Very frequently cytophores with several old nucleoli imbedded in different regions of their cytoplasm, indicating that fragmentation has occurred, show all their nuclei at the periphery as in the case of spermatids formed by mitosis. Since I have never found first spermatocytic mitoses and fragmentation occurring in the same cytophore and since the nucleolus disappears before mitosis it seems probable that the presence of the old nucleoli which stain very characteristically and are readily recognizable is sufficient to identify a particular cytophore and its spermatid nuclei, for such, I believe we may call them, at least so far as appearance goes, as the result of fragmentation.

Fig. 39 (Pl. XIV.) is probably a stage in the migration to the periphery of spermatid nuclei formed by fragmentation. At the right of the figure one of the old nucleoli, itself near the periphery, and a part of an old nuclear cavity containing a few granules are visible: another lies near the middle of the cytophore and still others were found in the same cytophore in other sections. Some of the nuclei have already reached the periphery of the cytophore and the cytoplasm about them bulges from its surface. Most of these nuclei are more or less hemispherical with convex surface toward the periphery and a space adjoins the flattened side: this condition is probably connected with the migration of the nuclei toward the periphery. Indeed, it is not impossible that this migration may not be a bodily movement through the cytoplasm of the cytophore, but rather the continued formation of new nuclear membrane on the peripheral side and its continued disappearance on the proximal side. In any case the change of position is probably a "tactic" reaction of some sort.

In two testes cytophores have been found containing nuclei intermediate in size between those of the spermatocytes at the time of fragmentation and those of spermatids, but which appeared as if preparing for fragmentation. Fig. 40 shows one of these cases. These two groups may possibly be cases of secondary fragmentation. Occasionally as in Fig. 30, *A* (Pl. XIII.), the nuclei arising by fragmentation are unusually large and perhaps

undergo fragmentation again. At any rate such cases are rare and not of fundamental importance. In one case a mitotic spindle was observed in a cytophore whose nuclei had apparently arisen by fragmentation since two nucleoli were present and the nuclei were not at the periphery (Fig. 41, Pl. XIV.). This would appear to be a case where a nucleus resulting from fragmentation divides mitotically afterward: it might, however, be a case where a spermatocyte undergoing mitosis had fused with a group undergoing fragmentation: if that were the case the size of the spindle would seem to identify it as the second spermatocytic division. But whatever its interpretation, this, too, is clearly an exceptional case. It is probable that a large proportion of the nuclear substance of the spermatocyte passes into the cytoplasm at the time of fragmentation. It may be that a kind of reduction is accomplished in this manner.

The relative frequency of fragmentation and mitosis seems to vary in different chains, proglottids and testes. In some chains the spermatogenetic mitoses were rarely seen except in the older proglottids, yet spermatozoa were produced in the younger proglottids as abundantly apparently as elsewhere. In some other cases mitosis is more frequent. From examination of the testes one gains the impression that the mitoses are not in any case sufficiently numerous to account for the large number of spermatids and spermatozoa formed. I regard it as at least probable that spermatozoa are produced from the "spermatid" nuclei which arise by fragmentation, as well as even those which arise by mitosis. As will appear in a later section, some cells undergo degeneration in almost all or all testes and it is of course impossible to prove that these particular spermatid nuclei which arise by fragmentation do not undergo degeneration. Still, developing spermatozoa have been found on cytophores containing the old spermatocyte nucleoli.

VI. *The Formation of the Spermatozoön from the Spermatid.*

In consequence of the difficulty of observation of details in these exceedingly minute structures, which is farther increased by the massing together of the spermatids in cytophores and the condensation of these in later stages, it has not been possible to reach positive conclusions on all points. It can scarcely be

doubted from my own observations as well as those of others that the development of the spermatozoön in these forms differs in certain respects from the typical method. Although the greatest caution has been observed throughout, the observations are given with a certain reserve, because it was impossible to attain complete certainty on many points and because they are not in accord with commonly accepted opinions regarding the development of the spermatozoön. I believe, however, that a careful investigation of spermatogenesis in the cestodes will prove of interest.

Except in the early stages when the spermatid nuclei produced mitotically appear in pairs about the cytophore (Fig. 42, Pl. XIV.) and those arising by fragmentation of the spermatocytes are massed in the interior of the cytophore (Figs. 34–36, Pl. XIII.) there is no certain criterion for distinguishing the two kinds. The presence of old nucleoli in a cytophore render it probable that all or a part of the spermatid nuclei of that cytophore have arisen by fragmentation, but beyond this no means of identifying the nuclei of different origin has been discovered.

The following account concerns the spermatids without respect to origin. If my conclusions are correct, however, many of these may be the results of fragmentation. As was noted above, developing spermatozoa not different in appearance from others have often been found on cytophores containing the old nucleoli.

Figs. 42 and 43 (Pl. XIV.) show the newly formed spermatid nuclei after mitosis. In Fig. 43, from *M. planissima*, five chromatin granules are distinctly visible in each of the nuclei. These may represent five chromosomes: it is possible that the spermatocytes of this species contained only five dyads (Fig. 16, *A*, Pl. XI.) and that the cases where a larger number seemed to be present (Figs. 16, *B*, 16, *C*, Pl. XI.) were only earlier stages before the chromatin had become massed in the dyads.

After the formation of the spermatid nuclei their peripheral position on the cytophore becomes more and more marked until finally each is borne on a short peduncle or stalk of cytoplasm (Fig. 44, Pl. XIV.). The cytophores differ greatly in size according as they were formed from a single spermatocyte or a larger number: in fact in older testes single isolated spermatids

are sometimes found which have apparently become entirely separated from the cytophore.

The spermatid nuclei contain at this time only a few very distinct deeply staining granules (some of the nuclei in Fig. 44, Pl. XIV.): in cases where extraction is carried to extremes only two granules, one at the peripheral end of the nucleus, the other near the middle or at one side of the nucleus (some of the nuclei in Fig. 44, Pl. XIV.). The peripheral granule is closely applied to the nuclear membrane, so closely indeed that it is often difficult to determine whether it is inside or outside the nucleus. In some cases, however, it is clearly inside the nucleus (Fig. 44, Pl. XIV.) and this is probably its position in all cases.

The first visible step in the formation of the spermatozoön is the appearance at the periphery of the cytoplasm peripheral to the nucleus of a minute deeply staining granule. In position this granule corresponds to the peripheral centrosome which enters the middle piece in the spermatozoa of many other forms. It has been impossible in consequence of the small size of these cells to obtain any data regarding its origin in this case. If the spermatids arising by fragmentation do produce spermatozoa the question as to its origin in those cases is of some interest. This peripheral body which apparently lies in contact with the border of the cytoplasm appears to be connected by a very delicate cytoplasmic strand or fiber with the granule at the peripheral end of the nucleus (Figs. 44 and 45, Pl. XIV.). Whether there is another cytoplasmic granule in contact with or near the nucleus corresponding in position to the other centrosome of other forms could not be determined. From the peripheral granule in the nucleus the delicate fiber appears to continue through the nucleus usually to the second granule (some of the nuclei in Fig. 44, also Fig. 45, Pl. XIV.). This continuation of the fiber within the nucleus has been a matter of the most careful examination and I can say regarding it only that I have seen it in the nuclei of practically every cytophore examined and under the most various conditions of fixation and staining so that if present methods of technique permit trustworthy conclusions in regard to such matters its existence seems beyond doubt. The figures exaggerate its distinctness to some extent. It does not stain as

deeply as the granules themselves but this is very likely due to its smaller diameter.

The next step is the formation of the tail which appears first as a delicate thread extending from the granule at the border of the cytoplasm (Fig. 46, Pl. XIV.).

Figs. 47, *A*–47, *E* (Pl. XIV.) show the developing spermatozoa after different methods of fixation and staining : Fig. 47, *A*, is from *M. expansa* after sublimate and Delafield's hæmatoxylin ; Fig. 47, *B*, *M. expansa* after sublimate and iron-hæmatoxylin ; Fig. 47, *C*, *M. planissima*, after chrom-oxalic and iron-hæmatoxylin ; Fig. 47, *D*, *M. expansa*, after Hermann and iron-hæmatoxylin. Fig. 47, *E*, is from *M. planissima* after sublimate and iron-hæmatoxylin, but with extraction stopped at an earlier stage. One interesting point in this figure as compared with the others is the much larger size of the peripheral cytoplasmic granule and the fiber connecting it with the nucleus — an excellent illustration of the uncertainty attending the use of iron-hæmatoxylin.

The tail of the spermatozoön grows to a very great length. Fresh spermatozoa obtained by teasing living proglottids in indifferent fluids are 0.3–0.4 mm. in length. Most or all the tails arising from one cytophore usually lie parallel in the testis, and since their length is much greater than the diameter of the testis they become coiled in the spaces between the cells or along the wall of the testis. The tail is very delicate and without visible differentiation in structure.

As regards the formation of the head of the spermatozoön *Moniezia* does not seem to agree with other species described. At least I know of no other case in which the sperm-head, if it can be called a head, is formed in the manner described below.

In my study of spermatogenesis I was for a long time puzzled by the fact that all of the sperm nuclei appeared to degenerate after the tails were formed. Masses like Fig. 49 (Pl. XV.) consisting of degenerating nuclei and condensed cytophore cytoplasm can be found in every older testis. At first I concluded that these were probably the spermatids formed by fragmentation which began the development of spermatozoa but were unable to complete it. But another feature made the matter still

more puzzling. The most careful examination, under varied conditions of fixation and staining, of spermatozoa in the male ducts and in the seminal receptacle of the female ducts, which becomes greatly distended with them at a certain stage, failed absolutely to reveal the existence of a head differing in appearance from the tail. The examination and staining of fresh spermatozoa from the seminal receptacle and ducts of living proglottids led to the same result. The spermatozoa appeared as very long thread-like structures perhaps slightly larger at one end than at the other but without the least trace of a physically or chemically differentiated head.

Then the question arose as to whether the eggs were actually fertilized by these spermatozoa. As will be described in the following paper, the spermatozoa were found entering the eggs as these passed the opening from the seminal receptacle on their way to the uterus, and nuclei which could be nothing else than male pronuclei unless these eggs differ from other known cases in their maturation and fertilization stages were found. Returning to the developing spermatozoa the most careful study was made of the various stages and especially of the masses like Fig. 49 (Pl. XV.) which were apparently undergoing degeneration. It is very difficult to distinguish details in these masses for they stain more deeply as they condense and the nuclei especially become more or less filled with deeply staining granules and masses. In the course of time certain apparently favorable cases were found some of which are shown in Figs. 48, *A*–48, *C* (Pl. XV.). These seem to indicate that the "head" of the spermatozoön, *i. e.*, the part arising from the nucleus is formed from the two nuclear granules, the peripheral and the other which may be central or proximal, together with the connecting strand, and furthermore, that when degeneration of the other parts of the nucleus begins the spermatozoön is set free. Figs. 48, *A*–48, *C*, show examples of the early stages of nuclear degeneration in sperm cytophores. In Fig. 48, *C*, the spermatozoön head is apparently in the act of escaping from the degenerating nucleus. The peripheral portion of the nuclear membrane has disappeared but the peripheral nuclear granule is still recognizable. Figs. 48, *A*, and 48, *B*, are apparently somewhat earlier stages in

which the nuclear membrane is still intact. I am forced therefore to the conclusion that only a part of the nucleus is concerned in the formation of the sperm-head, the remainder undergoing degeneration. Fig. 49 (Pl. XV.) represents a cytophore after condensation. For the sake of clearness only a few of the nuclei which cover the surface of the mass are represented as they appear, the others being indicated by dotted lines. In several cases what seems to be the sperm-head is visible in the degenerating nucleus.

Fig. 50 (Pl. XV.) represents a case in which the spermatozoa are apparently just separating from the cytoplasm of the cytophore which contains the deeply staining remains of the nuclei. In this case I convinced myself that these were the anterior ends of the spermatozoa, by following the tails throughout their whole length in the testis. The diameter of the ends shown in the figure was distinctly though only slightly greater than that of the other ends, but the change in diameter is very gradual.

In the free spermatozoa no differentation in staining of the head-region is visible. The whole spermatozoön stains uniformly and less intensely than the nuclear granules or masses of earlier stages. In a few cases I believed I had distinguished slight traces of the two nuclear granules in the fully developed spermatozoön but these observations were so doubtful that no figures are given.

In the fresh spermatozoa obtained by teasing in indifferent fluids no visible head and no movement was ever observed. An examination of the bibliography of the subject afforded scanty results. So far as I have been able to determine no full account of the spermatogenesis of the cestodes exists. Among the older papers several give brief descriptions of the formation of the spermatozoa but these are either very incomplete or incorrect in consequence of the technique employed and need not be reviewed in detail. In one point, however, the early observations agree fairly well: the head of the spermatozoön is described and figured as exceedingly minute or is said to be absent. Sommer and Landois[1] in describing the testes of *Bothriocephalus latus* mention spermatozoa bearing at one end "ein kleines, stark lichtbrechendes Köpfchen."

[1] Sommer and Landois, "Ueber den Bau der geschlechtsreifen Glieder von *Bothriocephalus latus* Bremser," *Zeitschr. f. wiss. Zoöl.*, Bd. XXII., 1872.

Two years later Salensky[1] states that in *Amphilina* the nuclei disappear completely in the formation of the spermatozoa and that "die Kerne bei der Bildung der Spermatozoen keine Rolle spielen." Regarding the fully formed spermatozoa he says: "Die Fäden sind sehr lang, ungefahr 0.27 mm. und an einen Ende etwas gekrummt. Diese Krümmung soll aber nicht als Köpfchen angesehen werden, indem die spermatozoen in ihrer ganzen Länge gleich dick sind."

As regards *Tænia mediocanellata* and *Tænia solium* Sommer[2] speaks of the bundles of spermatozoa which hang from certain large cells (in reality the cytophores) and "mit ihren äusserst feinen, glänzenden Köpfchen noch in Zellenprotoplasma stecken." These "Köpfchen" are probably the nuclear granules which present this appearance in unstained or slightly stained preparations, the nuclear membrane not being clearly visible. "Zwischen diesen Samenfäden producirenden Zellen findet man gleichzeitig im Hodenkörperchen kleine Anhäufungen freier, heller, scharf contourirter und bläschenförmiger Kerne. Einzelne derselben haben an ihrem Grenzrande noch Spuren von Protoplasma in welchen mit seinem glänzenden punctförmigen Köpfchen ein Samenfädchen haftet." These masses are perhaps degenerating cytophores. In another paragraph he describes the formation of the large multinucleate cells which give rise to the spermatozoa and says: "An der Peripherie dieser grossen Zellen geht von irgend einer Stelle die Bildung der Samenfäden aus. Letztere entstehen lediglich aus dem Protoplasma der Zelle; eine Betheiligung der Kerne dabei findet nicht statt. In demselben Maasse wie mit der Bildung der Samenfäden das Protoplasma der Zelle schwindet, werden die eingelagerten Kerne frei, erscheinen dann schärfer berandet wie früher, etwas aufgebläht oder gequollen, homogen und wasserhell, dann fallen sie zusammen, collabiren, wie wenn sie einen flussigen Inhalt entleert hatten und gehen zu grunde, oder werden, wenn sich inzwischen Samengänge ge-

[1] Salensky, "Ueber den Bau und die Entwickelungsgeschichte der *Amphilina*, G. Wagen (*Monostomum foliacium* Reed)," *Zeitschr. f. wiss. Zool.*, Bd. XXIV., 1874.

[2] Sommer, "Ueber den Bau und die Entwickelung der Geschlechtsorgane von *Tænia mediocanellata* (Küchenmeister) und *Tænia solium* (Linné)," *Zeitschr. f. wiss. Zool.*, Bd. XXIV., 1874.

bildet haben mit den Samenfäden fortgespielt." Sommer's preparations were obtained by maceration and teasing and without staining. As described, the fate of the nuclei does not differ very widely from that described in the present paper except for the fact that Sommer failed to observe that any portion of the nucleus took part in the formation of the spermatozoön. Considering the methods employed this failure is not strange.

Moniez[1] describes the formation of large multinucleate cells and the protrusion from their surface of the nuclei which are united with the body of the cell by pedicels (these are evidently the spermatids on the cytophore). He continues as follows: "Ces nouvelles formations qui rayonnent de la cellule-mere sont les vrais spermatozoides: leur flagellum se forme à la partie périphérique, tandis qu'ils sont encore fixé par l'autre extrémité; c'est apres qu'ils se sont détachés que leur tete s'atrophie comme l'on sait." These facts he describes as common to a number of species among them *Tænia expansa, i. e., Moniezia expansa* as it is now known.

In describing the spermatozoa of *Tænia saginata* Leuckart[2] speaks of the "freilich kaum ausgezeichneten" head.

From all of these observations it is evident that where a distinct head is visible it is exceedingly minute and the observations of Salensky, Sommer, and Moniez seem to indicate that the spermatozoa of several species are without visible heads. The description of the collapse and degeneration of the nuclei by Sommer and the mention of atrophy of the head by Moniez appear to be somewhat closely in line with my own observations. But until other species have been examined with the aid of present cytological methods general conclusions are impossible. I am convinced, however, that if the spermatozoa of *Moniezia* possessed distinct, visibly differentiated heads I should have seen them in some cases at least. Comparative study of other species will undoubtedly prove of interest.

VII. *The Degeneration of Cell-Groups in the Testis.*

During almost the whole period of existence of the testis groups of cells undergo degeneration from time to time. Cells in any

[1] Moniez, "Sur les Spermatozoides des Cestodes," *Comptes Rendus*, 1878.

[2] Leuckart, "Die Parasiten des Menschen," 1879–1886.

stage of development from the spermatogonia to the developing spermatozoa except during the spireme stage are subject to this fate. The proportion of degenerating cell-groups varies greatly in different chains, proglottids and testes. In some chains only one or two cases of degeneration preceding the first appearance of the spireme stage have been observed. In others degenerating groups are found in almost every testis in the spireme period. During the earlier stages of the process the degenerating cells form rounded masses: later these break up and become distributed through the testis and are apparently absorbed by the cytoplasm of other cells. While it is impossible to assign positively a definite reason for this degeneration I am inclined to believe that it results from differences in physiological condition which may in turn be correlated with differences in nutrition. Careful examination of regions of rapid growth in many forms often shows a certain proportion of cells which are undergoing degeneration. Undoubtedly in such regions the intensity of certain stimuli or conditions carries some cells beyond the point where physiological equilibrium can be regained and they degenerate, serving perhaps as food for the others. There can be little doubt that the testis is a region of this sort. The great variation in the frequency of degeneration in different chains may indicate that it is connected with nutritive conditions. Apparently more cells are produced than can be sustained and some are eliminated.

The fact that no case of degeneration beginning during the spireme stage has been observed may be of some interest. It is not improbable that this stage is relatively independent of external conditions, *i. e.*, that a cell having entered this stage is capable of completing it without the intervention of external factors. To judge from appearances this stage is a readjustment or the establishment of a new condition of equilibrium in the cell and it may represent a reaction from previously existing conditions which have disturbed the previous equilibrium of the cell. There can be little doubt that in many respects the life of the cell possesses a cyclical character. One complex of processes or reactions continues until it brings about a reversal in reaction or initiates a different complex, etc.

That this degeneration has any connection with amitosis is

extremely improbable. In no case has a whole testis been found undergoing degeneration, yet in all the testes most of the divisions before spermatogenesis proper were amitotic and in the great majority the first divisions certainly were amitotic. As was suggested in the preceding section degeneration of cell-groups in post-spireme stages may be connected with the fragmentation of spermatocyte nuclei though this seems improbable, and moreover, it does not explain degeneration in pre-spireme stages. I believe, though I see no way of demonstrating it, that the method of origin of the cell-groups in the testis has no connection with their degeneration.

The degenerating cell-groups vary greatly in appearance according to the stage at which degeneration begins and the different stages of degeneration itself. In many cases, though not always, it is possible to determine from the appearance of the degenerating mass approximately the stage at which degeneration began. In some cases cells in the same stage of development undergo two different processes of degeneration.

Some of the characteristic forms and stages of degenerating cell groups are shown in the following figures : in these figures no attempt has been made to represent the cytoplasmic background. This varies somewhat in density and staining in different cases. Vacuoles and spaces are indicated by broken lines. The method of reproduction exaggerates the depth of shade in the more deeply staining portions. Fig. 51 (Pl. XV.) shows a small group of cells from a young testis in the first stages of degeneration. The first evidence of degeneration in these cases is a condensation of the cytoplasm and a massing together of the nuclei, and the degenerating group becomes quite distinct from other cells, usually lying in a space. Fig. 52 (Pl. XV.) shows a later stage of this form of degeneration ; the nucleoli increase in size and stain very deeply, the nuclear membrane becomes indistinct, and the whole mass stains more intensely. Later, as shown in Fig. 53 (Pl. XV.), the mass breaks up into irregular deeply staining fragments and strands which are distributed through the testis and are often found in the cytoplasm of other cells surrounded by small vacuoles ; a few of these fragments in the cytoplasm are shown in the figure.

Fig. 54 (Pl. XV.) represents a form of degeneration in cells in prespireme stages which sometimes occurs in old testes. Here the nuclei form irregular densely staining masses and finally the whole breaks up and is absorbed.

Fig. 55 (Pl. XV.) shows degeneration of a group of spermatid nuclei in a cytophore. The deeply staining granules and masses in the nucleus increase in number, the nuclear membrane breaks down, and the granules are distributed through the cytoplasm. In Fig. 56 (Pl. XV.) another form of spermatid degeneration is seen and a later stage in Fig. 57 (Pl. XV.). Fig. 58 (Pl. XV.) represents a still later stage: vacuoles usually containing a single granule still indicate the position of the nuclei; the mass stains only very faintly at this stage and seems to decrease gradually in size until finally it becomes imbedded in a cytophore and gradually disappears.

Fig. 59 (Pl. XVI.) represents a form of degeneration of the spermatids which usually occurs only after the spermatozoa have begun to develop. About the periphery of each nucleus a large amount of deeply staining substance develops and appears to flow toward the center of the cytophore. In Fig. 60 (Pl. XVI.) is shown a later stage of this form of degeneration. Here the cytoplasm stains rather more deeply than that of the normal cytophore, the deeply staining substance has disappeared entirely from the peripheral regions except in a few radiating strands and the positions of the nuclei are indicated only by vacuoles. In still later stages (Fig. 61, Pl. XVI.) the deeply staining substance gradually breaks up into granules (Fig. 64, *k*, Pl. XVI.), loses its staining power and finally disappears, and the whole cytophore becomes highly vacuolated, breaks up into irregular masses and shreds (Fig. 64 *l*, Pl. XVI.) and is apparently absorbed.

Other modifications of the process of degeneration are occasionally seen but these are the principal ones. The apparent variation which these processes exhibit is of some interest as indicating that differences in the processes of degeneration like differences in development are undoubtedly determined by differences in the condition of the cells or of the environment. At present, however, even a surmise as to the nature of these differences is of little value.

VIII. *The Full-Grown Testis.*

The testis continues to increase in size for a considerable time after spermatogenesis begins. Only a part of the spermatogonia enter the spireme stage at any one time, the others continuing to divide amitotically. After the appearance of the spermatogenetic divisions in a testis, I have never seen a case of mitosis in the spermatogonia, but amitoses are frequent. Figs. 62, *A*, 62, *B* (Pl. XVI.) represent groups of spermatogonia in full grown testes. In the same testes all stages of spermatogenesis and fully developed spermatozoa may be found. At this period the spermatogonia are usually found in small groups near the periphery. Figs. 63, *A*–63, *D* (Pl. XVI.), show cases of amitosis in spermatogonia from full grown testes, including nearly all the modifications of the process observed.

In Fig. 64 (Pl. XVI.), one half of a full-grown testis is shown on a scale half as large as that of the other figures. The different stages shown are as follows: at *a* is a group of spermatogonia still in the prespireme stage and showing one amitosis; *b* shows the earliest stages of the spireme, *c*, *c*, two groups with fully-developed spireme, while at *d* some cells are preparing for the first spermatocytic mitosis; at *e* is a cell in which the dyads are formed, part of a group which appears in adjoining sections; *f*, *f*, are cytophores with spermatid nuclei and developing spermatozoa; *g*, is a cytophore in which degeneration of the nuclei has begun, but the spermatozoa are still attached; at *h* is seen part of a bundle of free spermatozoa which can be followed in other sections; *k*, *k*, represent two degenerating cytophores in which the nuclei have already vanished: the shreds of cytoplasm and the debris from earlier cytophores are indicated at *l*. Although this one section does not show all the stages in the history of the cells, it serves to indicate the promiscuous distribution of different stages.

IX. *Conclusion.*

The point of chief importance in the present paper is the fact that typical mitosis and amitosis may appear together and apparently under identical conditions in the development of the male as well as of the female germ cells. The relative frequency of

the two forms of division varies in different chains, proglottids and regions. Observations and experiments to be described later will show very clearly, however, that amitosis as well as mitosis is an important factor in growth, not only in *Moniezia* but in many other forms and that in some cases at least either form of division may be changed into the other by altering the conditions.

These facts are of considerable importance as bearing upon certain hypotheses regarding the significance of the chromosomes. At present it seems improbable that the views held by certain authors regarding the individuality of the chromosomes can be reconciled with them. Extended discussion is, however, postponed until other facts have been presented.

The most important features in the development of the male germ cells in *Moniezia* are as follows :

The testes apparently arise from cells which are already differentiated as muscle-cells, as well as from other cells of the parenchyma. The earlier divisions are almost entirely amitotic, mitosis being rarely seen.

The growth of the testis up to the time when spermatogenesis proper begins is almost wholly by amitotic division. In the full-grown testis the remaining spermatogonia still continue to divide amitotically. After the spireme stage the spermatocytes follow two very different lines of development. In some of them typical dyads are formed and the two usual spermatogenetic mitoses follow : the spermatid nuclei are usually situated about the periphery of large masses of cytoplasm, cytophores formed by fusion of the spermatocytic cytoplasm, but may be isolated.

In the other spermatocytes the nucleus increases in size, the spireme breaks up into granules and masses and loses most of its staining power, the old nuclear membrane disappears, and new nuclear membranes form about small fragments of the chromatin : each spermatocyte may give rise to several small nuclei : in appearence these nuclei are indistinguishable from the spermatid nuclei produced mitotically. When first formed they are massed in groups in the interior of the cytophore about spaces which indicate the former positions of the spermatocyte nuclei. The nucleolus does not take part in this fragmentation but remains in the cytoplasm of the cytophore for some time. The nuclei thus

formed gradually make their way to the periphery of the cytophore and probably give rise to spermatozoa, though this cannot be demonstrated with absolute certainty.

Apparently only a part of the nucleus is involved in the formation of the anterior end of the spermatozoön in which no "head" is visible. The sperm-head is apparently represented by two granules in the nucleus, one peripheral, one more or less nearly central and a less deeply staining fiber which connects them, these being in most cases the only deeply staining portions of the nucleus. When development of the spermatozoön is completed the nuclear portion is apparently set free from the remainder of the nucleus by the degeneration of the latter.

Groups of cells in all stages of development except the spireme stage are frequently attacked by degenerative processes probably because of insufficient nutrition or exhaustion.

HULL ZOÖLOGICAL LABORATORY,
UNIVERSITY OF CHICAGO,
September, 1906.

EXPLANATION OF PLATES.

PLATE XI.

FIG. 14. *A*, *B*, the dyads grouped about the periphery of the nucleus. *M. expansa.*

FIG. 15. Dyads irregularly disposed in nucleus. *M. expansa.*

FIG. 16. *A–C*, dyads. *M. planissima.*

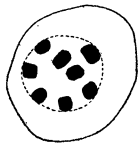
FIG. 17. Two dyads more highly magnified.

FIGS. 18, 19, 20, 21. Different stages of first spermatocytic mitosis.

FIG. 22. Two dyads in metaphase, more highly magnified.

FIG. 23. After the first spermatocytic division.

FIG. 24. Probably resting nuclei after first spermatocytic mitosis.



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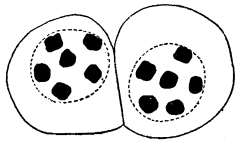

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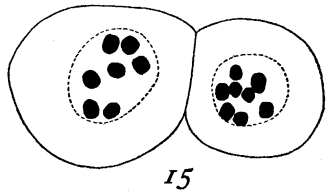

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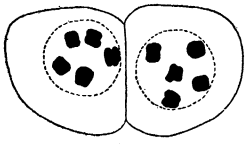

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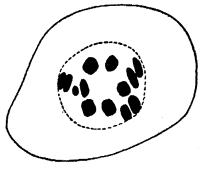

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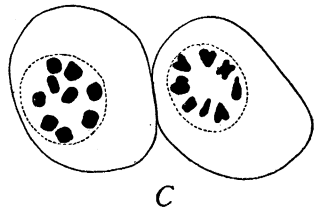

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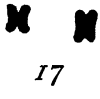

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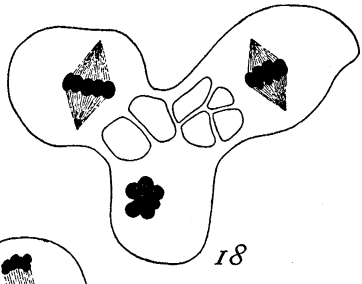

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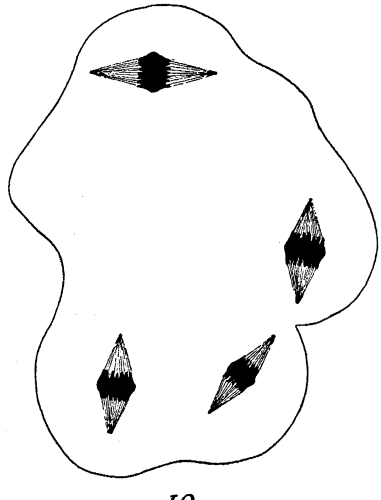

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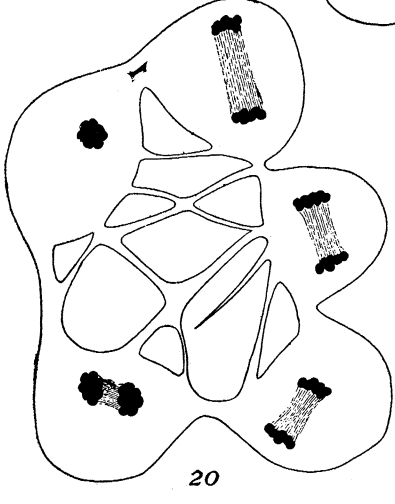

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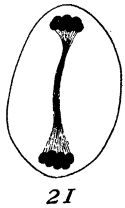

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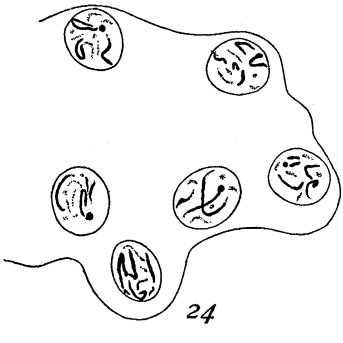

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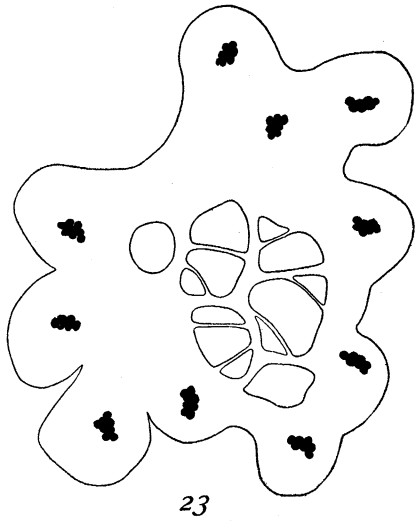

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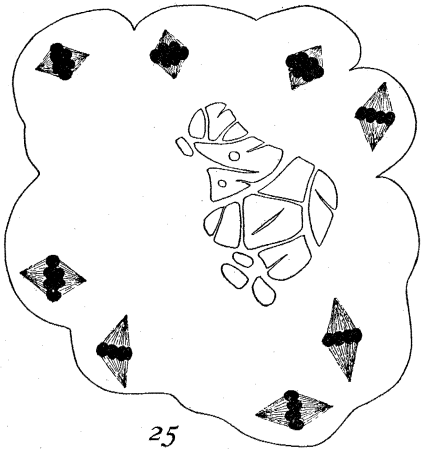
PLATE XII.

FIGS. 25, 26. The second spermatocytic mitosis.

FIG. 27. *A–D*, early stages of fragmentation of nuclei of first spermatocytes.

FIG. 28. Fragmentation. One small nucleus forming.

FIG. 29. *A–E*, the formation of nuclei by fragmentation.



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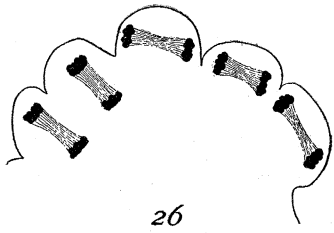

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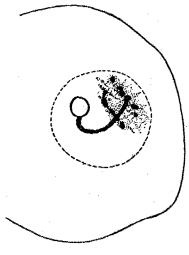

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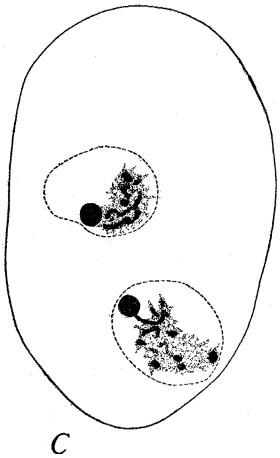

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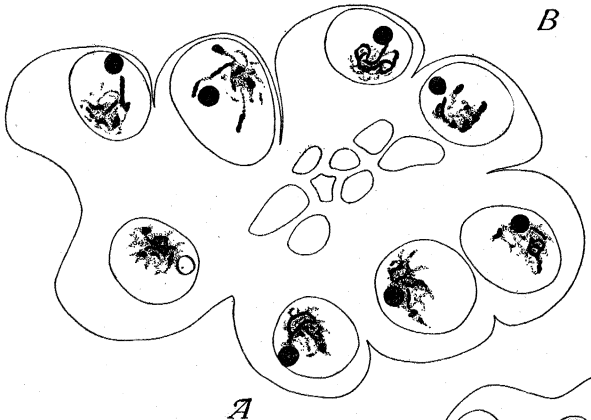

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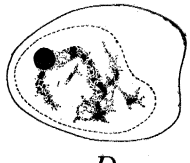

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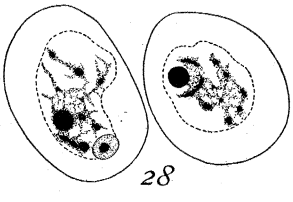

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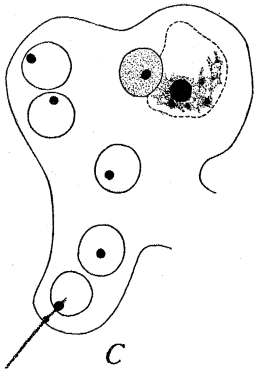

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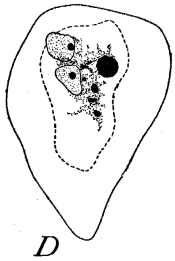

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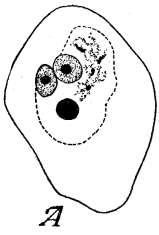

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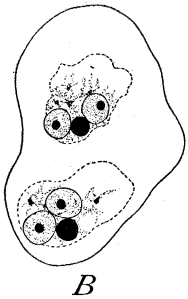

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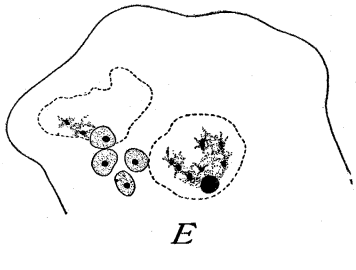

E

PLATE XIII.

FIG. 30. *A–C*, the formation of nuclei by fragmentation.

FIGS. 31–36. Nuclei and old nucleoli in the cytophores after fragmentation.

FIG. 37. Nuclei and old nucleolus after fragmentation with spireme stages adjoining.

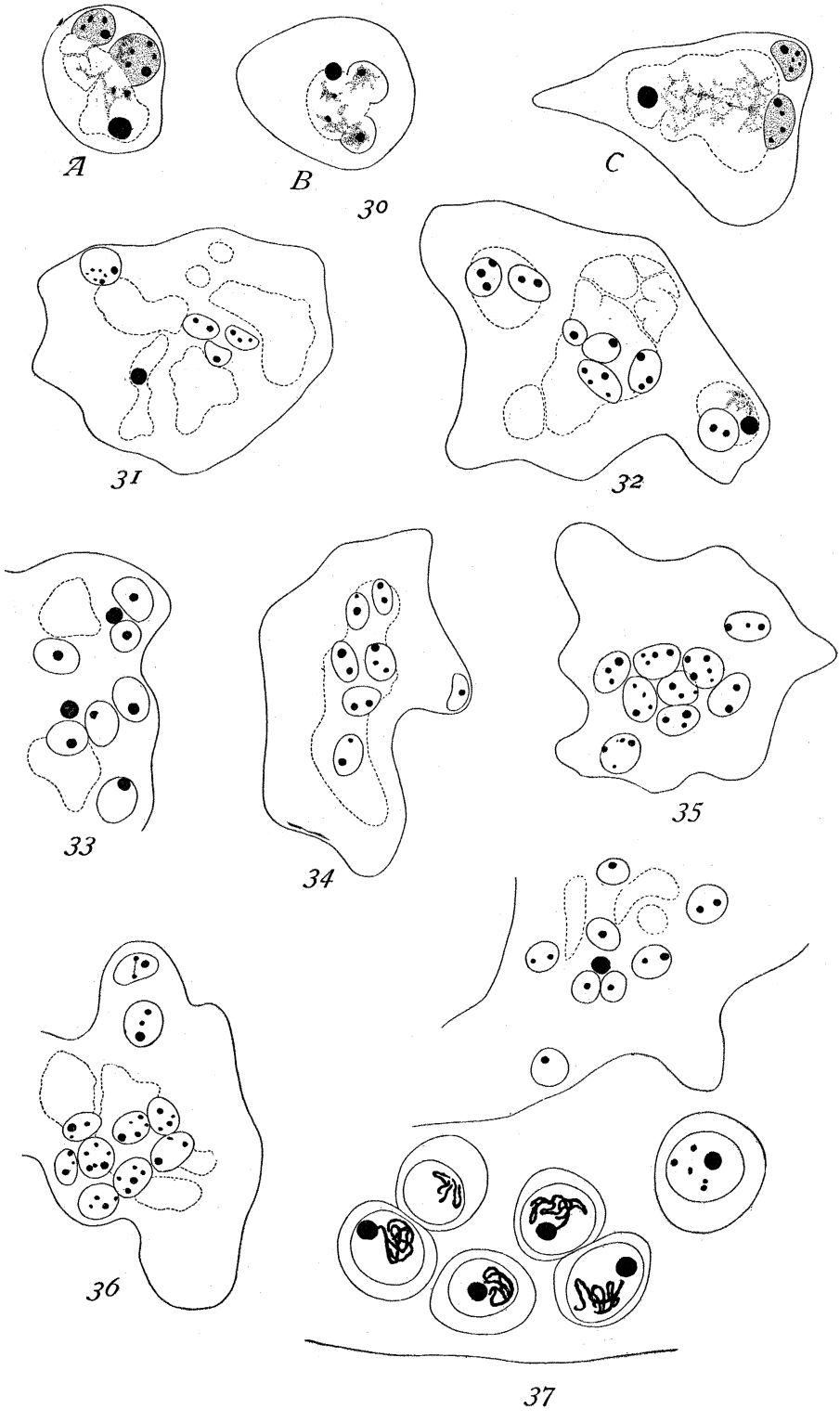
A
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PLATE XIV.

FIG. 38. Nuclei and old nucleolus after fragmentation with other stages adjoining.

FIG. 39. Probably migration of nuclei to periphery after fragmentation.

FIG. 40. Possible case of secondary fragmentation.

FIG. 41. A case of mitosis in a cytophore which was probably formed by fragmentation.

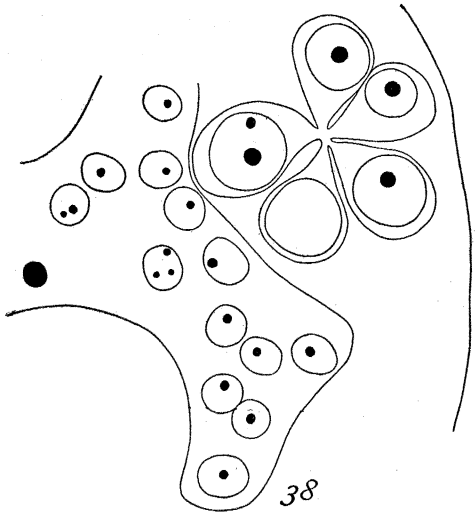
FIG. 42. Spermatid nuclei formed by mitosis.

FIG. 43. Spermatid nuclei.

FIGS. 44 and 45. Early stages in the development of the spermatozoa.

FIG. 46. Development of the spermatozoa.

FIG. 47. *A–E*, development of the spermatozoa after different methods of fixation and staining; *A*, *M. expansa*, sublimate and Delafield's hæmatoxylin; *B*, *M. expansa*, sublimate and iron-hæmatoxylin; *C*, *M. planissima*, chrom-oxalic and iron-hæmatoxylin; *D*, *M. expansa*, Hermann and iron-hæmatoxylin; *E*, *M. planissima*, sublimate and iron-hæmatoxylin, extraction stopped at an early stage.



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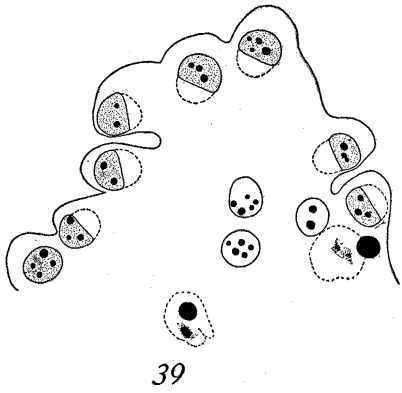

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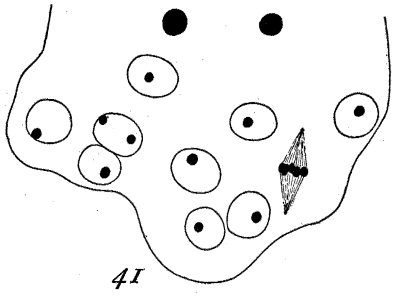

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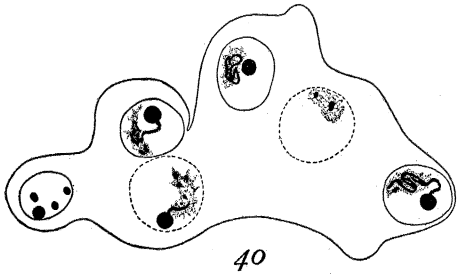

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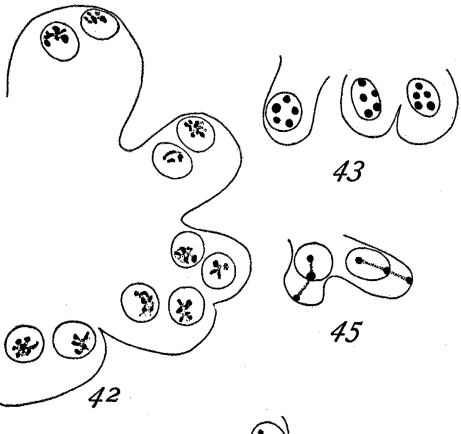

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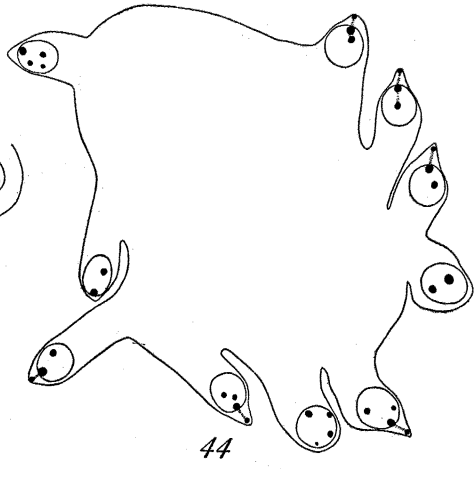

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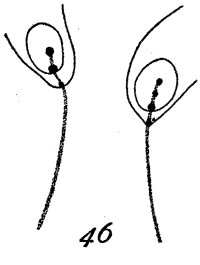

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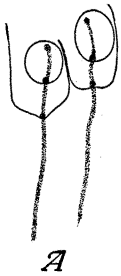


A

B

47 C

D

E

PLATE XV.

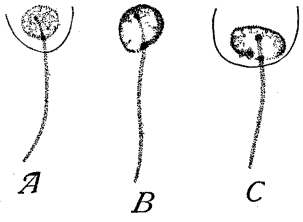
FIG. 48, *A–C.* The spermatozoön and the degenerating portions of the spermatid nucleus.

FIG. 49. A cytophore with spermatozoa and degenerating spermatid nuclei.

FIG. 50. Spermatozoa becoming free from degenerating cytophore.

FIGS. 51–54. Degeneration of cell groups in prespireme stages.

FIGS. 55–58. Degeneration of cell groups in spermatid stage.



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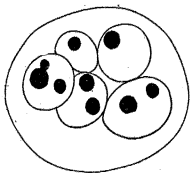

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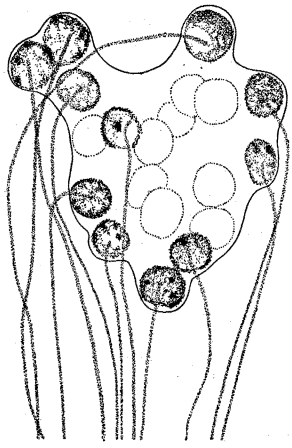

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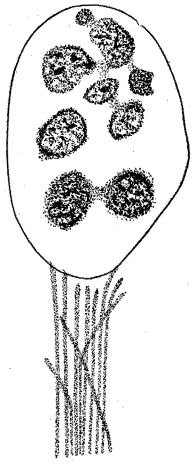

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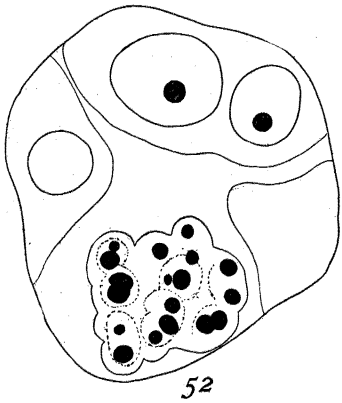

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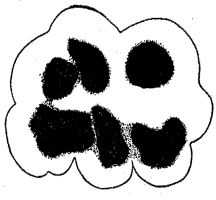

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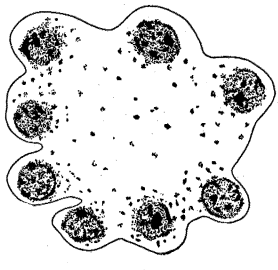

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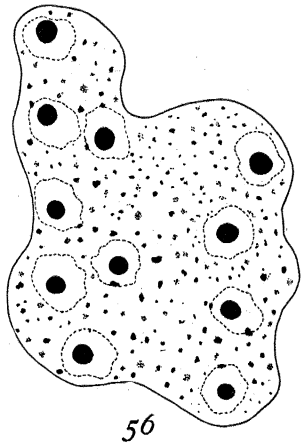

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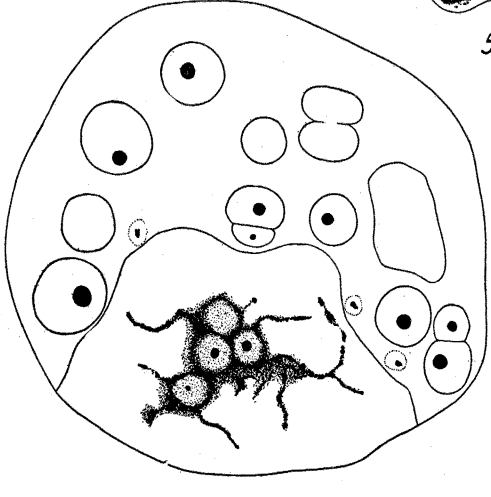

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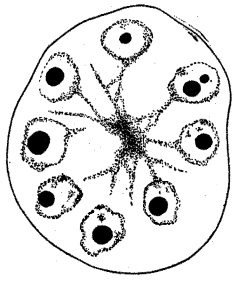

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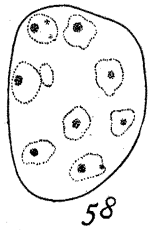


58

PLATE XVI.

FIGS. 59, 60. One form of degeneration of cytophores after development of spermatozoa.

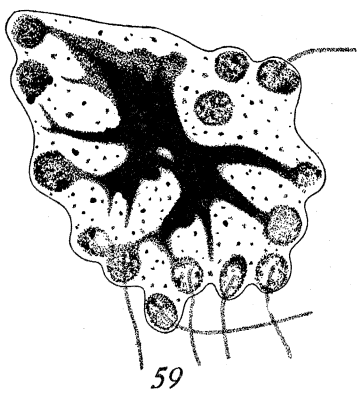
FIG. 61. Late stage of degenerating cytophore.

FIGS. 62, *A*, *B*; 63, *A–D*. Spermatogonia dividing amitotically; from full grown testes.

FIG. 64. Half of full grown testis; scale one half that of other figures; *a*, spermatogonia; *b*, early stages of spireme formation; *c*, *c*, spireme stage; *d*, preparation for first spermatocytic mitosis; *e*, dyads before first spermatocytic mitosis; *f*, *f*, cytophores with spermatids; *g*, cytophore with degenerating nuclei and spermatozoa; *h*, free spermatozoa; *k*, *k*, degenerating cytophores; *l*, shreds of protoplasm from earlier cytophores which have undergone degeneration.

The following figures are from *Moniezia expansa:* 1 *B*, 1 *D*, 3 *A*, 3 *B*, 3 *C*, 4 *B*, 4 *C*, 9 *D*, 10 *A*, 11 *A*, 11 *B*, 12 *A*, 13 *A*, 13 *B*, 14 *A*, 14 *B*, 15, 17, 18, 20, 21, 23, 26, 27 *D*, 28, 29 *E*, 30 *A*, 30 *C*, 31, 32, 34, 35, 36, 37, 38, 39, 40, 42, 44, 45, 46 47 *A*, 47 *B*, 47 *D*, 48 *A*, 48 *B*, 48 *C*, 49, 51, 55, 57, 58, 59, 60, 61, 62 *A*, 62 *B*, 63 *A*, 63 *B*, 63 *C*, 63 *D*, 64.

The others are from *M. planissima*.



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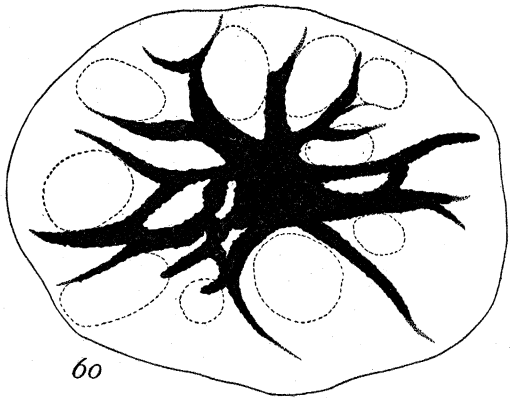

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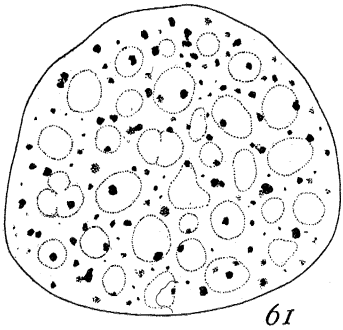

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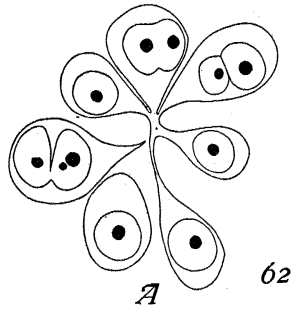
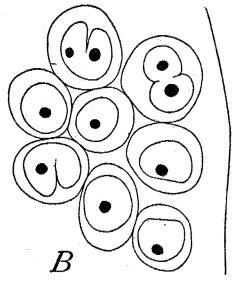


A

62



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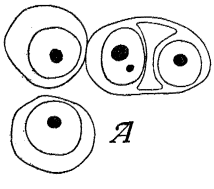

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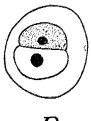

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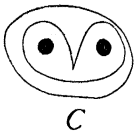

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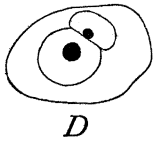

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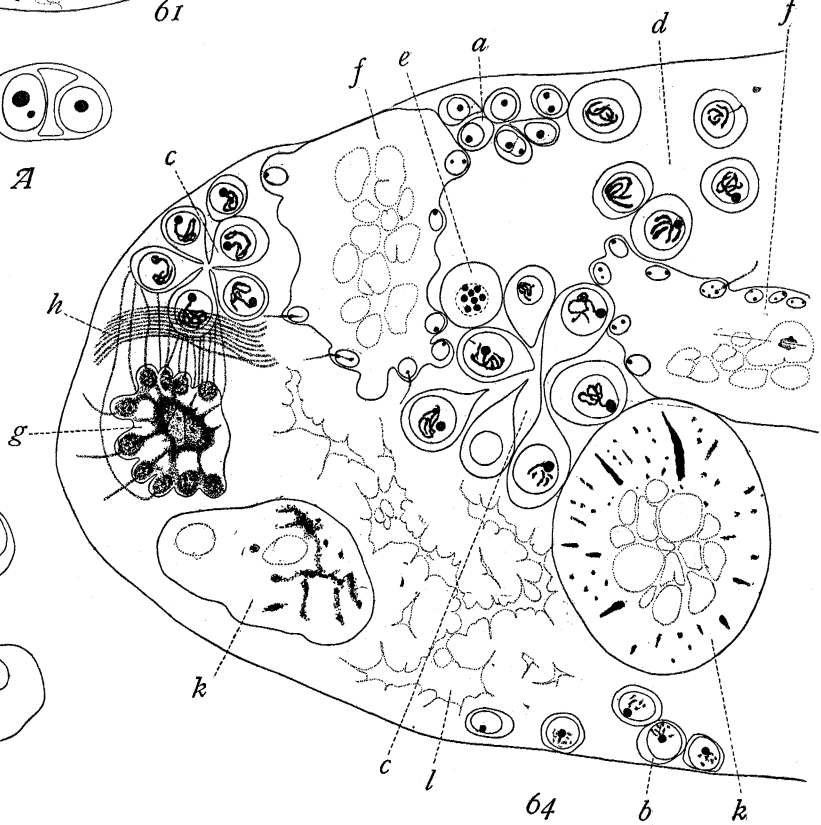
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